HEATED ELECTROCHEMICAL CELL

RELATED APPLICATIONS

This application is a continuation of copending PCT Application No. PCT/AU99/00152, filed on 11 March 1999, which claimed priority from Australian Patent Application No. PP2388, filed on 12 March 1998.

TECHNICAL FIELD

This invention relates to a method and apparatus for measuring the concentration of an analyte in solution.

The invention will be described with particular reference to the measurement of the concentration of glucose in blood but is not limited to that use and has general application for the measurement of analytes other than glucose and for solutions other than blood samples.

BACKGROUND ART

Persons who suffer from diabetes routinely check their blood glucose concentration and there is a need for simple, reliable and inexpensive means to facilitate such routine testing.

In a common method for conducting the tests, a blood sample is combined with an enzyme for example glucose dehydrogenase ("GDH"); the GDH oxidises glucose and in the process becomes reduced. An oxidising mediator, for example ferricyanide, is allowed to react with the reduced GDH returning the GDH to its initial form and producing ferrocyanide in the process. The concentration of ferrocyanide produced is then sensed for example electrochemically or spectroscopically to produce a signal which can be interpreted to give an estimate of the glucose concentration in the sample.

In our co-pending applications PCT/AU96/00723 and PCT/AU96/00724 (the disclosures of which are incorporated herein by reference) there are described methods and apparatus suitable for electrochemically determining the concentration of glucose in blood by electrochemical measurement.

A preferred method for accurately determining the concentration of an analyte is to react all the analyte present in the sample with reagents that produce a species that can be sensed. This requires that the reaction of the analyte go to completion.

For reaction of GDH with glucose to go to substantial completion typically requires several minutes. This is thought to be due to the time required for the glucose to diffuse out from glucose-containing cells of the blood. As this length of time is unacceptably long for the market, it is more usual to measure the glucose concentration over a shorter period, for example 20-30 seconds and accept a less accurate response or apply a factor to estimate the glucose concentration by kinetic extrapolation for example as outlined in co-pending application PCT/AU96/00723. This expedient shortens the time of the test but can lead to loss of precision of the result.

It is an object of the present invention to provide a method and apparatus which avoids or ameliorates the above-discussed deficiencies in the prior art.

DESCRIPTION OF THE INVENTION

According to one aspect the invention consists in a method for determining the concentration of an analyte in a sample comprising the steps of:

heating the sample in a disposable test cell; and

measuring the concentration of the analyte or the concentration of a species representative thereof in the sample at a predetermined point on a reaction profile by means that are substantially independent of the temperature of the sample in the test cell.

Those skilled in the art will understand the term "reaction profile" as used herein to mean the relationship of one reaction variable to another. Often, for example, the reaction profile illustrates the change of concentration of a species with respect to time.

Such a profile can provide a skilled addressee with both qualitative and quantitative information, including information as to whether a reaction system has achieved a steady state.

Preferably, the predetermined point on the reaction profile is a steady state, and the species representative of the concentration of the analyte is a mediator, for instance an enzyme mediator.

In one embodiment of the invention the sample is heated by an exothermic reaction produced upon contact of the sample with a suitable reagent or reagents.

In a second embodiment of the invention the sample is heated electrically, for example by means of a current applied to resistive elements associated with the measuring means.

In a highly preferred embodiment the measuring means is an electrochemical cell of the kind described in co-pending applications PCT/AU96/00723 and PCT/AU96/00724 and the sample is heated by application of an alternating voltage signal between electrodes of the sensor.

According to a second aspect the invention consists in an electrochemical cell comprising a spacer pierced by an aperture which defines a cell wall, a first metal electrode on one side of the spacer extending over one side of the aperture, a second metal electrode on the other side of the spacer extending over the side of the aperture opposite the first electrode, means for admitting a sample to the cell volume defined between the electrodes and the cell wall, and means for heating a sample contained within the cell.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention will now be more particularly described by way of example only with reference to the accompanying drawings wherein:

Figure 1 shows schematically a sensor strip according to the invention in a cross-section taken longitudinally through the midline of the sensor strip.

Figure 2 shows the results of tests conducted in accordance with one embodiment of the present invention for blood samples with varying haematocrits and glucose concentrations.

Figure 3 shows the results of tests conducted in accordance with another embodiment of the present invention for blood samples with varying haematocrits and glucose concentrations.

BEST MODE FOR CARRYING OUT THE INVENTION

In preferred embodiments of the method of the invention, glucose concentration is measured using an electrochemical cell of the kind described in PCT/AU96/00723 and/or PCT/AU96/00724 (our co-pending applications). The method of measurement described in those applications utilises an algorithm which enables the value of the diffusion coefficient of the redox mediator to be calculated and the concentration of reduced mediator to be determined in a manner which is substantially independent of sample temperature. The method therein described is different from prior art methods which measure Cottrell current at known times after application of a potential. The present invention differs in that the sample is heated.

In a first embodiment of the present method the blood sample is heated prior to and/or during conduct of the electrochemical measurement by means of an exothermic

reaction. In the first embodiment a reagent that liberates heat on contact with blood is contained within the sensor cell. Examples of such reagents are salts which give out heat when they dissolve such as aluminium chloride, lithium halide salts, lithium sulphate, magnesium halide salts and magnesium sulphate. Another class of reagents which would be suitable are those with two components which liberate heat upon mixing. These two components would be placed in separate locations in the sensor during fabrication, for example on coatings upon opposite internal cell walls and are deployed such that when a sample is introduced into the sensor at least one of the components dissolves and then comes into contact with the second component. Upon contact the two components react to liberate heat. The reagents used to generate the heat must not adversely effect the function of the other active elements in the sensor. For instance, they must not corrode the electrode materials, denature an enzyme if present, or adversely interact with any mediator that may be present. Upon introducing a sample of blood into the sensor heat is liberated and the temperature of the blood sample is raised. This facilitates reaction of the glucose with the GDH and since the measurement of ferrocyanide concentration is temperature independent an accurate assessment of glucose concentration can be made in a much shorter time than would otherwise be possible.

Less preferably, the heat generating reagent can be added after the sample is admitted to the cell.

Preferably the sample temperature is raised by from 5 to 15°C, for example from 20°C to 30°C or 35°C within a period of 2 to 10 seconds. The temperature peak is desirably reached within 2-5 seconds.

A second embodiment of the invention employs a cell in which an electrically resistive element is incorporated. The sample may then be electrically heated by passing a current through the resistive element. For example, with reference to figure 1 there is shown an electrochemical sensor comprising a plastic substrate 1 bearing a first electrode 2 (for example a sputtered layer of gold), a separator layer 3 having a circular aperture punched out which defines a cell volume 10 bounded on one cylindrical face by first electrode 2. The opposite face of cylindrical cell 10 is covered by a second electrode layer 4 (for example a sputter coating of palladium) which in this case is carried by a rubber or plastic layer 5. A metal foil layer 6 provides electrical contact to a resistive bridge 9 formed in the rubber or plastic layer 5. An insulating layer 7 for example of plastic provides insulation against heat loss through the metal foil. An aperture 8 in layer 7 provides for electrical contact with metal foil layer 6. Resistive bridge 9 is formed for example from carbon particles impregnated into the rubber or plastic of layer 5 at a loading and of a geometry such as to give a suitable electrical resistance between metal foil 6 and electrode layer 4. This method has the advantage of concentrating the heating effect adjacent the cell. Resistive heating elements may be fabricated by other means for example by coating an electrically conducting substrate with an electrically insulating layer which can be made partially conductive in particular regions if desired for example by exposure to particular chemicals and light.

When using a cell according to the second embodiment the sample is admitted to the cell, a potential is applied across the resistive element, and after the required amount of heat has been generated the potential across the resistive element is interrupted and after

an optional wait time a potential is applied between the first electrode and second electrode to perform the electrochemical assay of the analyte.

Alternatively the potential across the resistive element can be maintained during the assay of the analyte at its initial level or at a lower level sufficient to substantially maintain the sample temperature at the desired level.

In another embodiment, the means for applying the potential to the resistive element is such that the current flowing through the resistive element is monitored and the potential automatically adjusted so as to maintain the required power output. This heats the sample in a reproducible fashion, even if the resistance of the resistive element varies from one sensor to the next. Furthermore, the power level required can be adjusted on the basis of the ambient temperature measured by a separate sensor. The leads to a more reproducible sample temperature being reached over a range of ambient temperature at which the sensor is being used.

In a third embodiment of the invention the sample is heated simply by applying an alternating voltage signal between the working and counter-electrodes of a sensor, for example, of the kind described in our co-pending applications. If this alternating voltage signal has a correct frequency and amplitude it will heat the sample while still allowing an accurate determination of the analyte to be subsequently made by the sensor. Because the voltage signal is alternating any reaction that occurs during one half voltage cycle is reversed during the second half of that cycle, resulting in no net change but in the dissipation of energy that will appear as heat in the sample. This is particularly applicable to sensors of the type disclosed in our abovementioned co-pending patent

applications where any small changes that may occur in the cell are quickly removed after interruption of the alternating potential as the cell relaxes back to its initial stage.

When using cells such as described in our co-pending applications the sample volumes are very small and heating can be achieved with low energy input.

EXAMPLES OF HEATED STRIP EXPERIMENTS

Example 1

Disposable test strips of the type described in PCT/AU96/00724 were heated by placing a metal bar, heated to 50°C, in contact with the sample receiving area of the strip. Whole blood samples were introduced into the sample receiving area of the strip and 13 seconds allowed for the glucose present in the sample to react with the sensor reagents. Current was then collected for ten seconds and analyzed according to the methods described in PCT/AU96/00723. The results of these tests for blood samples with haematocrits of 67.5%, 49.5% and 20% and glucose concentrations between 2.5 mM and 30 mM are shown in figure 2.

Example 2

Disposable test strips of the type described in PCT/AU96/00724 were modified by adhering a heater element to the base of the strip, beneath the sample receiving area. The heater element was fabricated by sputtering two parallel low resistance metallic tracks onto a polyester substrate and then sputtering a thin, resistive metallic track at right angles to the low resistance metallic tracks, such that the resistive metallic track contacted both of the parallel low resistance tracks. This heater was then glued to the base of the disposable test strip using an adhesive, such that the resistive track was positioned directly beneath and facing the sample receiving area on the strip.

The parallel low resistance tracks protruded from the end of the strip and provided electrical contacts for a power supply to power the heater. The power supply for the heater consisted of a battery and a variable resistor, which could be adjusted to vary the rate of heating. Whole blood samples were introduced into the sample receiving area of the strip and 20 seconds allowed for the glucose present in the sample to react with the sensor reagents. Current was then collected for ten seconds and analyzed according to the methods described in PCT/AU96/00723. The results of these tests for blood samples with haematocrits of 65%, 46% and 20% and glucose concentrations between 2.8 mM and 32.5 mM are shown in figure 3.

Although the invention has been herein described with reference to electrochemical methods for measuring glucose concentration in blood it will be appreciated that the method may also be applied utilising suitable spectroscopic or other measuring methods and to samples other than blood and to analytes other than glucose.